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Ecotoxicity evaluation of Cu- and Fe-CNT complexes based on the activity of bacterial bioluminescence and seed germination

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ABSTRACT

The toxic effects of the composites of Fe^0 and Cu^0 with different percentages of CNTs were examined based on the activity of bacterial bioluminescence and seed germination. In terms of the EC_{50} values, the toxic effects of Cu^0 on bacterial bioluminescence and seed germination were approximately 2 and 180 times greater than that of Fe^0, respectively. The toxicity increased with increasing CNT content in the Cu-CNT mixtures for both organisms, whereas opposite results were observed with Fe-CNT mixtures. The mean toxic effects of Cu-CNT (6%) were approximately 1.3–1.4 times greater than that of Cu-CNT (0%), whereas the toxic effects of Fe-CNT (6%) were approximately 2.1–2.5 times lower than that of Fe-CNT (0%) for both the bioluminescence activity and seed germination. The causes of this phenomenon are unclear at this point. More research will be needed to elucidate the mechanism of the toxicity of nano-mixture materials and the causes of the different patterns of toxicity with Cu- and Fe-CNT mixtures.

Introduction

Nanotechnology has emerged as an enabling technology with high potential impact on virtually all fields of mankind. Estimates suggested that by 2015 nanotechnology will have a trillion-plus dollar global economic impact (Ge et al., 2012). Berman (2016) also reported that nanotechnology will bring improvements by addressing environmental problems using advanced nanotechnology by 2025. The implementation of unique materials and devices ranging from electronics to engineered tissues is one of the rapidly growing fields of nanotechnology (Shvedova et al., 2003). Two advanced nanomaterials that are being used increasingly for different technologies are carbon nanotubes (CNTs) and graphene. CNTs are well-ordered, high aspect ratio allotropes of carbon. CNTs have attracted considerable interest from both scientists and industry because their unique atomic configuration, mechanical, optical, electronic properties, high aspect ratios (*e.g.*, like fibers), strength, and remarkable physical properties (Smart et al., 2006). Current global multi-wall CNTs production capacity is estimated to be 13,996 tons (Zhao et al., 2017). Carbon nanotube metal matrix composites are an emerging class of new materials that are being developed because of the high tensile strength and electrical conductivity of carbon nanotube materials.

Powder metallurgy processing to prepare nanocrystalline CNT composite powders with Cu and Fe have attracted considerable attention in the next generation automobile industry to manufacture the desired advanced automotive

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components because of their promising mechanical, electrical, and thermal properties. Any exposure of humans to CNTs would pose considerable danger to human health. Therefore, understanding and ensuring the safety of nanomaterials are of huge importance to the tremendous commercial applications of nanotechnology (Foldvari and Bagonluri, 2008; Tang et al., 2012). Safety evaluation of nanomaterials should consider their behaviors in various aspects, including their interactions with proteins, DNA, lipids, membranes, organelles, cells, tissues, biological fluids, and even the dissolution of metal constituents (Zhao et al., 2008; Nel et al., 2009; Liu et al., 2013). There are different ways, in which the human body can be exposed to nanomaterials, including penetration through the skin, ingestion, inhalation, and injection (Zhao and Liu, 2012).

A great effort in recent years has been performed to elucidate the mechanism of nanotoxicity in living organisms and cells. For this purpose, scientists and researchers have examined the toxicity of CNTs in several cell lines (Wang et al., 2011; Pichardo et al., 2012). Their efforts suggest that various factors, including functioning, dimensions, and characteristics, can determine the toxicity of CNTs, including environmental factors, such as temperature, humidity, and barometric pressure, affecting rates of consumption and even the occurrence of some toxic agents (Horie et al., 2012). Recently, *in vivo* toxicology studies, by focusing on multiple organ systems, have proved that CNTs can cause a toxic response within these multiple organ systems. Research into the ecotoxicity of nanomaterials has demonstrated different levels of toxicity depending on the bioassays (Brunner et al., 2006; Soto et al., 2006). TiO_2 and ZnO nanomaterials, which are used widely in sun protection products as well as self-cleaning coatings, have been shown to have toxic effects that can inhibit the growth of microalgae, crustaceans, and bacteria, while opposite results have been reported by other investigators (Serpone et al., 2007; Heinlaan et al., 2008; Aruoja et al., 2009). Ecotoxic bioassays using various test organisms (bacteria, algae, protozoa, plants, and fish) and their metabolic processes have gained widespread attention for environmental contaminants (Banks and Schultz, 2005). Knowledge of each test organism's sensitivity is important for evaluating the contaminant toxicity levels. Among these processes, seed germination and bacterial bioluminescence were adopted because of their high sensitivity, simplicity, *etc.* Plant toxicity assays are particularly relevant when the phytotoxic contaminants of nanomaterials are present in soil (Boutin et al., 2004). Among plant processes, seed germination studies are considered short-term because they assess the rapid response to acute toxicity. The root and shoot elongation test is one of the simplest short-term methods used in environmental biomonitoring (Di Salvatore et al., 2008). Assays based on bacterial bioluminescence are a time-saving and cost-effective test that are used widely as a reproducible and sensitive screening method for determining the acute toxicity of different sample types (Wang et al., 2002).

In this study, composites of Fe^0 and Cu^0 with different percentages of CNTs, which are currently used in laboratories and the automobile industry without any restriction by environmental law, were tested. The ecotoxic effects of composites of Fe and Cu with CNTs were evaluated based on the activity of bacterial bioluminescence and seed germination.

1. Materials and methods

1.1. Preparation of metals-CNT mixtures

CNTs were synthesized using a catalytic CVD method and then dispersed in distilled water at concentration of 5 wt.% CNT using a Sonosmasher. Carboxymethyl cellulose (CMC) was used as the dispersant with a 9 sec working interval and 1 sec off continuously, totaling 6 hr in dispersion for the finally dispersed product. Copper powder with a particle size of up to 63 μm was purchased from Markin Metal Powders Ltd. (UK). Iron powders with a particle size of up to 150 μm were purchased from Hoganas Ltd. Each metal powder was placed into a milling chamber with the CNTs solution for the attrition ball milling process using SUS-316 L balls, 5 mm in diameter, as the milling media. Ball milling was proceeded at 300 r/min for 1 hr. The milled solution was then dried in an oven for 24 hr to produce the final milled and mixed metal-CNTs powder. Four different conditions of the mixture samples were prepared according to the CNT concentrations: 0, 1%, 3%, and 6% (W/W).

1.2. Toxicity test of metals-CNT mixtures on bioluminescence activity

The toxicity of the metal-CNT mixtures was measured based on a bioluminescence activity of the *Escherichia coli* DH5α strain RB1436, harboring a variant of the pUCD615 plasmid (obtained from R. Burlage, Concordia University, USA). This strain, which contains a constitutive promoter to express the *lux* genes, produces bioluminescence in a growing culture and is used to detect deleterious conditions that would cause a measureable decrease in bioluminescent output, such as those induced by metal-CNT mixtures. The RB 1436 strain was stored at −70°C until needed, at which time, it was grown overnight in Luria–Bertani^{ka} (LB^{ka}) medium at 27°C with shaking at 130 r/min. The culture was diluted 30-fold in LB^{ka} medium and allowed to grow until the optical density (OD_{600}) reached approximately 0.6. This culture was diluted appropriately with minimum salt medium to a final OD_{600} of 0.2 for the toxicity test (Ko and Kong, 2014). For the test, 1 mL of the diluted bacterial suspension was mixed with 9 mL of the sample, after which the bioluminescence activity was measured after 1 and 1.5 hr of incubation. The bioluminescence activity was measured using a Turner 20/20 luminometer (Turner Design, USA), which had a maximum detection limit of 9999 relative light units (RLU).

1.3. Toxicity test of metals-CNT mixture on seed germination

The seed (*Lactuca sativa* L.) produced and distributed by a commercial seed company (Nongwoo Bio., South Korea) were purchased from a local seed store. These particular seeds were employed in the test because the plants from which they were obtained are important food crops in the local region. Prior to the germination test, all seeds were surface-sterilized with an aqueous 3% H_2O_2 solution for 10 min and then rinsed with distilled water. Filter paper was then placed in a sterilized Petri dish and moistened with 5 mL of an aqueous solution